for providing mechanical support to the failing heart in these settings.
Methods: We retrospectively reviewed all patients who received a Thoratec Single-Lead-Vented-Electric LVAD at our institution between August 1990 and February 2003. Twenty-five patients with viral myocarditis were identified. Twenty-five patients whose primary indication for LVAD was coronary artery disease (CAD) were randomly selected from the same database to serve as a control group. Variables analyzed included patient demographics, duration of LVAD support, preoperative white blood cell (WBC) count and erythrocyte sedimentation rate (ESR) values, percent explanted, success rate of bridge to transplantation, and post-transplant survival rates.
Results: The VM group was younger than (35.88+/-16.43 years vs. 58.88+/-4.30 years) ($p<0.01$) and consisted of a greater proportion of female patients than (36% vs. 8%) ($p=0.02$) the CAD group. Duration of LVAD support, preoperative WBC and ESR values, and percent explanted were similar between the two groups. Bridge success rates and post-transplant survival rates were also comparable (64% transplanted in VM, 60% transplanted in CAD ($p=0.86$); 1- and 5-year post-transplant survival rates of 86.67% and 72.80% in VM, 71.43% and 62.50% in CAD, respectively ($p=0.34$)).
Conclusions: These findings suggest that despite the variable clinical course of VM and the potential to rapidly progress to end-stage heart failure, LVAD implantation in these patients yields outcomes similar to those receiving LVADs for CAD. Device support permits decompression of the dilated ventricle, facilitating myocardial recovery and the likelihood of bridging successfully to transplant or explant.

POSTER SESSION

1051 Cardiac Transplantation: Cellular Mechanisms and Rejection

Sunday, March 07, 2004, 3:00 p.m.-5:00 p.m.
Morial Convention Center, Hall G
Presentation Hour: 4:00 p.m.-5:00 p.m.

1051-127 Molecular Pathways of Cardiac Allograft Dysfunction Independent of Acute Cellular Rejection

Mandeep R. Mehra, Patricia A. Uber, Myung H. Park, Robert Scott, Hector O. Ventura, Jay G. Wohlgemuth, James G. Prentice, Ochsner Clinic, New Orleans, LA, Expression Diagnostics, South San Francisco, CA

Background: The biology of allograft dysfunction independent of cellular rejection remains poorly understood. B-type natriuretic peptide (BNP) can serve as a sensitive marker of graft dysfunction despite normal systolic function. This investigation was designed to evaluate gene expression (GE) patterns associated with graft dysfunction and to identify underlying molecular pathways independent of cellular rejection.
Methods: Cardiac allograft recipients were prospectively enrolled as part of The Cardiac Allograft Rejection Gene Expression Observational (CARGO) multi-center study. Subjects were followed at each post-transplant visit with biopsy (read by 3 pathologists blinded to clinical data), whole blood BNP, echocardiography and hemodynamics. GE profiles of circulating cells were evaluated using leukocyte microarrays with > 8,000 genes and validated with real-time PCR (RT-PCR).
Results: 42 subjects were followed for two years. For 342 encounters the median BNP level was 190 pg/ml. Levels differed significantly as a function of gender and ethnicity (higher in women and black Americans, $p < 0.05$). BNP levels were elevated in those with Grade 3A rejection (n=9) compared to Grade 0 (n=35, $p < 0.003$) but lacked specificity for acute rejection. GE profiles of patients with elevated BNP levels ($\geq$295 pg/ml) compared to those with lower levels ($\leq$182 pg/ml, n=27) identified 25 genes correlated to BNP ($p=0.035$). The genes were associated with granulocyte and monocyte lineages and included elastases, adherence receptors, metalloproteinases and cytokine receptors. They were distinct from genes correlated to acute cellular rejection using microarrays and RT-PCR in the multi-center study. For 35 patients, BNP levels were compared to quantitative results of a clinically validated 14 gene RT-PCR test for acute cellular rejection. No correlation was found.
Conclusions: Peripheral immune cell molecular pathways indicative of allograft dysfunction are associated with elements of innate immunity distinct from cellular immunity pathways. GE assays for acute rejection and assessment of graft function by BNP may be complementary for detection of the quiescent state in cardiac allograft recipients.

1051-128 Infant Norwood Patients Become Sensitized to Donor HLA Antigens but Not Tolerized to Incompatible Donor ABO Antigens Following Homograft Implantation

Natalia E. Lobach, Lisa Hornberger, Jeffrey F. Smallhorn, Anne I. Dipchand, Neal denHollander, Glen VanArsdell, Stacey M. Pollock-BarZiv, Lori J. West, Hospital for Sick Children, Toronto, ON, Canada, University of Toronto, Toronto, ON, Canada

Background: In heart transplantation, pre-transplant anti-HLA antibody (sensitization) increases risk of antibody-mediated rejection and other post-transplant problems. Tissue allografts (homografts) used for aortic arch reconstruction and blood products used in the Norwood procedure may cause HLA sensitization in infants, some of whom may need subsequent heart transplantation. This study aimed to determine the incidence of HLA sensitization after homograft implantation in infants.
Methods: In this cross-sectional analysis, patients who underwent the Norwood procedure in infancy were tested (n=11) post-surgery and compared with control patients who received blood products during infant cardiac surgery without allograft placement (n=4). HLA sensitization was detected using Panel Reactive Antibody screening tests (PRA) and ELISA assays to detect antibody to HLA Class I & II antigens. Development of anti-blood group antibodies (isoagglutinins) was also investigated in study patients by reverse blood typing.
Results: Median age at surgery was 6 days (0-62 d) in allograft recipients, and 9 days (0-41 d) in controls. Median age at testing was 10 months (4mo-4yrs) in allograft recipients and 4 years (2-6yrs) in controls. 91% of allograft recipients were sensitized (PRA$\geq$10%), with 82% highly sensitized (PRA$\geq$4/12); 0% of controls were sensitized. 91% of allograft recipients showed positive ELISA to HLA Class I & II antigens. Two allograft recipients have undergone transplantation. Their HLA antibodies were shown in antibody-specificity assays to be directed against the HLA type of their homograft donors. Anti-blood group antibodies developed normally, even in patients whose allografts were from ABO-incompatible donors.
Conclusions: HLA sensitization develops in infants following tissue allograft placement, but not after exposure to blood products. ABO incompatible allografts did not affect normal development of isoagglutinins. These results show divergent effects on the infant immune system by exposure to T-dependent vs. T-independent antigens, and have important implications for infants eventually needing heart transplantation after Norwood palliative surgery.

1051-129 Use of Quantitative Reverse Transcriptase-Polymerase Chain Reaction for Validation of Macrophage Inflammatory Protein-1β and Vascular Endothelium-Cadherin as Important Markers of Acute Rejection After Heart Transplantation

Ana L. Roussoulieres, Olivier Raisky, Lara Chalabreysse, George Dureau, Catherine Cerutti, Pascale Boissonnat, Laurent Sebbag, Jean-Paul Gare, Jean-François Obadia, Jean Ninet, Olivier Bastien, Françoise Thivolet-Bejui, John L. McGregor, Hôpital Cardiologique Louis Pradel, Lyon, France, Inserm U331, Lyon, France

Background: Some molecules are involved in acute rejection following heart transplantation (HT). We have identified by macroarrays and immunohistochemistry (IHC), in a murine model of heterotopic HT, a number of genes implicated in acute allograft rejection. In this study, the expression of two of these genes, MIP-1β and VE-Cadherin, was investigated by quantitative real time polymerase chain reaction (RT-PCR).
Methods: We have previously studied the expression profile of genes involved in acute rejection after heterotopic HT in a murine model. Hearts from Balb/c mice were transplanted heterotopically in the abdomen of Balb/c (isografts) and C57BL/6 mice (allografts). Total RNA was extracted from mice hearts that were not transplanted (NT), from isografts and allografts. Using the technique of macroarrays and IHC, we have shown that MIP-1β was over expressed and that VE-Cadherin was under expressed in the acute rejection group (allografts). To validate the macroarrays and immunohistochemical results, the mRNA copy numbers for MIP-1β and VE-Cadherin were determined in the 3 groups using quantitative RT-PCR and TaqMan technology. Levels of the gene transcripts between the 3 groups were compared using the Kruskal-Wallis test. Mann-Whitney U test was used when comparing between 2 groups. P values $\leq$0.05 were considered to indicate significant statistical differences.
Results: The results showed that MIP-1β and VE-Cadherin were differentially expressed between the 3 groups ($p=0,01$ and $p=0,009$ respectively) as observed in the macroarray data and IHC staining. The relative amount of MIP-1β was significantly increased in allografts compared to isografts and NT ($p=0,01$ and $p=0,02$ respectively). The relative amount of VE-Cadherin was significantly decreased in allografts compared to isografts and NT hearts ($p=0,05$ and $p=0,02$ respectively).
Conclusions: We have identified 2 genes (MIP-1β and VE-Cadherin) as markers of acute rejection after HT in a murine model. Several lines of evidence, obtained by macroarrays and validated by IHC and quantitative RT-PCR confirmed the above statement. Validated genes can be used as potential targets in acute rejection after HT.

1051-130 Inhibition of p38 Mitogen Activated Protein Kinase Mediates Endothelial Cell Survival During Cardiac Transplantation

Matthew R. Mulloy, Edwin Kwon, Davis C. Drinkwater, Jr., Heather E. Gallagher, Yenya Hu, Vanderbilt University Medical Center, Nashville, TN

Background: The hypothermic ischemic preservation required for cardiac transplantation exposes the donor heart to myocardial ischemia/reperfusion (I/R) injury upon implantation. p38 mitogen-activated protein kinase (MAPK) has been directly linked to increased apoptosis in models of myocardial I/R injury and its inhibition has significantly improved post-ischemic myocardial function in *in vivo* models. However, the intracellular signaling pathways responsible for these changes are not well determined. Additionally, the incorporation of p38 MAPK inhibitors into myocardial preservation solutions has yet to be examined. Here we hypothesize that the incorporation of the p38 MAPK inhibitor, SB239063, into University of Wisconsin (UW) preservation solution results in effective inhibition of TNF-α-induced p38 MAPK activation. The inhibition of p38 MAPK may play a key role in mediating endothelial cell survival through the activation of the pro-survival signals, AKT and ERK1/2.
Methods: Confluent cultured human umbilical vein endothelial cells (HUVEC) at 37°C are pre-incubated in cold (4°C) UW solution at 4°C with or without SB239063 (50μM, 4°C, 12hr). Cells are rewarmed and activated with TNF-α. Lysates are analyzed for p38, AKT, and ERK1/2 activities by Western blotting.
Results: 1) UW solution with SB239063 successfully inhibited TNF-α-induced p38 MAPK activation (n=3). 2) Inhibition of p38 MAPK produced an average upregulation of AKT activity of 42% and an average upregulation of ERK1/2 activity of 148% (n=3).
Conclusion: The p38 inhibitor, SB239063, has been effectively incorporated into UW solution to inhibit TNF-α-induced p38 MAPK activation in HUVEC. Inhibition of p38 MAPK upregulates the activities of the anti-apoptotic signals AKT and ERK1/2. These data suggest that p38 MAPK is a pro-apoptotic signal whose inhibition may represent a novel method to mitigate apoptosis and improve myocardial performance following cardiac transplantation.